Hardly any clones arising more than six bacterial diameters behind the expanding front fixed. Closer to the front, we found the probability of fixation of P- clones to be strongly increased compared to the probability for neutral P^+ clones to reach the front. P- bacteria were expelled from the P^+ microcolony. Due to high surface tension of P^+ colonies, P- bacteria accumulated at the front of expanding colonies. This could be confirmed by characterising spontaneous mutations from the P^+ to the P- state in range expansion assays.
We conclude that the reduced physical interaction between P- and P^+ cells, leads to an increased probability of fixation for P- gonococci within a microcolony. It is tempting to speculate that gonococci can shield their major antigen, the type IV pilus, by surrounding themselves with P- cells.

1884-Pos Board B614
Dissecting the Role of Ferrous Iron in Pseudomonas Aeruginosa Gene Regulation
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In our quest towards a predictive understanding of biological systems, we have combined quantitative models of gene regulation with careful experimental tests of model predictions in order to determine how promoter architecture dictates the output level of gene expression. We have applied this quantitative framework to understand how cells sense and respond to extracellular cues, using ferrous iron-mediated gene regulation in Pseudomonas aeruginosa as an important case study.
Upon recent discovery of a two-component system that specifically responds to ferrous iron, there is an emerging picture of the critical role of Fe(II) availability in the regulatory outputs of Pseudomonas aeruginosa. On the bench top Fe(II) is readily oxidized to Fe(III), however in infections such as in the lungs of cystic fibrosis patients, cells can encounter reducing environments containing Fe(II). Ferrous iron regulates genes related to both pathogenicity and biofilm formation. These pathways are also controlled by the quorum sensing systems and the availability of ferric iron. It is not yet clear why multiple pathways are used to sense extracellular iron or how the cell integrates information from these different pathways to effectively respond to changes in the availability and oxidation state of iron.
Using statistical mechanical models of gene regulation and the tools of synthetic biology, the rules of the ferrous iron regulon are beginning to emerge. We quantified how promoter architecture dictates the regulatory response to Fe(II). A quantitative understanding of how promoters encode a ferrous iron response enabled us to predict how Fe(II) influences global gene expression patterns. Some genes respond to both Fe(II) and additional regulatory inputs, such as other transcription factors or two-component systems. We dissected these multi-input promoters to understand how cells combine ferrous iron availability with other regulatory factors to make transcriptional decisions.

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Using a Transcriptional Network to Approach the Mechanism of Fungal Meningitis
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C. neoformans is the most common cause of fungal meningitis, but the molecular mechanisms of pathogenesis remain poorly understood. The pivotal pathogen transcriptional regulator Gat201 inhibits phagocytosis independently of the polysaccharide capsule and plays a central role in virulence (1). To define the corresponding transcriptional network, we performed chromatin immunoprecipitation and sequencing (ChIP-Seq) and expression profiling (RNA-Seq) experiments of Gat201 and two other transcription factors, also required for virulence, regulated by Gat201 that comprise a regulatory network intimately tied to C. neoformans' virulence. Within the set of genes whose promoters are bound by all three transcription factors assayed, there are three members of a six-member protein family in C. neoformans (Blp1-6), characterized by an N-terminal signal sequence and a double-psi beta barrel motif. One member of this family, Blp1, inhibits phagocytosis through an unknown mechanism (1). In addition, we found that the promoter of a peptide required for low-density growth of a C. neoformans tup1Δ mutant strain background is also bound by all three transcription factors (2). We are currently investigating the functional roles of these secreted proteins and their biochemical mechanisms of action.
(1) Chun CD, Brown JCS, and Madhani HD. (2011) A Major Role for Capsule-Independent Phagocytosis-Inhibitory Mechanisms in Mammalian Infection by Cryptococcus neoformans. Cell Host & Microbe, 9: 243-251.
(2) Lee H, Chang YC, Nardone G, Kwon-Chung KJ. (2007) TUP1 disruption in Cryptococcus neoformans uncovers a peptide-mediated density-dependent growth phenomenon that mimics quorum sensing. Molecular Microbiology 64(3): 591-601.

1886-Pos Board B616
Altering Stochastic Noise in Gene Expression for HIV Therapy
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HIV's ability to enter a transcriptionally dormant state and establish a reservoir of latently infected cells is the major barrier to eradicating HIV from infected patients. Significant efforts are aimed at reactivating latent HIV and purging the reservoir through targeting of molecular mechanisms implicated in the establishment of latency. These efforts to perturb the latent reservoir have faced substantial challenges. Our previous studies implicated stochastic noise (i.e. fluctuations) in an HIV transcriptional positive-feedback circuit as crucial for the establishment of HIV latency and predicted that perturbing noise would alter HIV latency (Weinberger et al. Cell 2005; Dar et al. PNAS 2012). Here, we demonstrate that manipulation of stochastic noise in HIV gene expression radically perturbs HIV latency. Screening a library of small-molecule drug compounds identified over 50 compounds that modulate noise in the HIV promoter without changing the promoter's mean expression level. Strikingly, the noise-modulating compounds synergize with conventional transcriptional activators and surpass current best-in-class reactivation cocktails, while maintaining greater cell viability. Thus, noise-modulating compounds may present an approach to perturb the stability of the latent state. More generally, stochastic noise may represent a new unexplored axis for drug discovery that allows enhanced control over cell-fate specification decisions, metastasis, and pathogen persistence phenotypes.

1887-Pos Board B617
Correlating Rat Basophil Leukemia Cell Activation with Interleukin 4 RNA Production using Single Molecule Fluorescence In-Situ Hybridization, Automated Super-Resolution Microscopy, and GPU-Enabled Image Analysis
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Single-molecule, single-cell studies of genetic expression have provided key insights into how cells respond to external stimuli. Direct single-cell measurements of individual bio-molecules, such as small RNA (sRNA) or messenger RNA (mRNA) transcripts, make it possible to quantify the heterogeneity in spatiotemporal responses of key signaling and regulatory processes. Critically, these investigations provide information on cellular fluctuations-information that is hidden by typical biochemical ensemble measurement approaches. Recent work has suggested that macroscopic cell properties, such as cell morphology, are correlated with gene expression. Here we present single-cell studies of a signal-activated gene network: Interleukin 4 (IL4) RNA production in rat basophil leukemia (RBL) cells during the allergic response. IL4 mRNA production has been closely linked to histamine release by RBL cells in ensemble measurements. To further investigate this regulatory network, we fluorescently label individual IL4 RNA transcripts in populations of RBL cells, subject to varying external stimuli. A custom super-resolution microscope and GPU-accelerated data analysis package are used to measure the number of fluorescently labeled IL4 transcripts in populations of RBL cells on a cell-by-cell basis. To test the hypothesis that RBL cell morphology may connected to IL4 production and histamine release, we analyze white light images of RBL cells and cross-reference cell morphology with IL4 RNA levels. We find that the activation of RBL cells, determined by white-light imaging, is well correlated with IL4 mRNA expression yet highly heterogeneous for certain stimuli.

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Extrinsic Noise Driven Phenotype Switching in a Self-Regulating Gene
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Analysis of complex gene regulation networks gives rise to a landscape of metastable phenotypic states for cells. Heterogeneity within a population arises due to infrequent noise-driven transitions of individual cells between nearby metastable states. While most previous works have focused on the role of intrinsic fluctuations in driving such transitions, in this work we investigate the role of extrinsic fluctuations. First, we develop an analytical framework to study the combined effect of intrinsic and extrinsic noise on a toy model of a Boolean regulated genetic switch. We then extend these ideas to a more biologically relevant model with a Hill-like regulatory function. Employing our theory and extensive Monte Carlo simulations, we show that extrinsic noise